Lipid droplets play an important role during obligate intracellular bacterial infections

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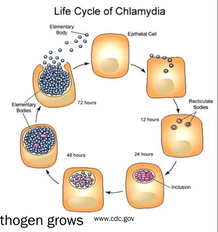
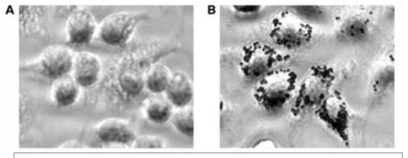
Chlamydia pneumoniae

Causative agent of sinusitis, pharyngitis, bronchitis, pneumonia, and linked to atherosclerosis.



Pathogenesis
- Life cycle similar to *C. trachomatis*
- Infects smooth muscle cells, endothelial cells of coronary arteries, macrophages, and adipocytes.
- Grows in the inclusion membrane
- Manipulates host mechanism by inducing expression of inflammatory cytokines, pro-coagulants, matrix metalloproteinase, and adhesion molecules

Lipid Droplets and *C. pneumoniae*
- *C. pneumoniae* is found around LDs of atherosclerotic plaques, amalgamate as pathogen grows
- **Atherosclerotic foam cell formation**
  - Down-regulates mRNA levels of PPAR-α and PPAR-γ, thus releasing inhibition of ACAT1 in macrophages and progression of macrophage foam cell of atherosclerotic lesions
  - Treatment with PPAR-α and PPAR-γ agonists significantly inhibits *C. pneumonia* induced foam cell formation. (Cheng et. al, Mei et. al)
  - Upregulates MAPK pathways to manipulate macrophage cholesterol metabolism, induce foam cell formation, and increase atherogenesis. (Cheng et. al)
- **Host lipolysis and lipid metabolism modulation**
  - Hijacks and reduces intracellular adipocyte FABP4, which affects lipid uptake, transportation, esterification, and β-oxidation of fatty acids, and HSL, which regulates lipid signal transduction. (Walenna et. al)
  - Upregulates SRA1, CD36, and ACAT1, which increases LDL uptake and accumulation of cholesterol and cholesterol esters in lipid droplets. (De Villiers et.al)
  - LXR and PPAR-α/PPAR-Y pathway activators can modify host cholesterol efflux through down regulation of ABCA1 and ABCG1. (Xu et. al)
  - Increases LDL-oxidation, total intracellular cholesterol, cholesterol esters, TAG levels, reduced cholesterol uptake, and decreases conversion to bile acids dyslypidemia. (Marangoni et. al)



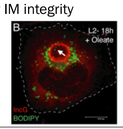
C. pneumoniae inducing foam cell formation and CE accumulation (A) Oil red-O stained macrophages treated with LDL (B) infection of *C. pneumoniae* in the presence of LDL for 24 hours (Cao, et. al)

Chlamydia trachomatis

Causative agent for trachoma, the second leading cause of blindness worldwide (serovars A-C) and the most common sexually transmitted infection (STI) worldwide (serovars D-K) which can lead to Pelvic Inflammatory Disease (PID), infertility, and ectopic pregnancy

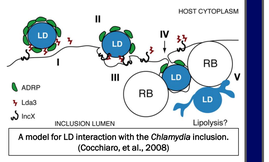
Pathogenesis:
- Exists in two forms: infectious, vegetative, spore-like elementary bodies (EBs) and non-infectious, metabolically active reticulate bodies (RBs)
- RBs replicate within the inclusion membrane (IM) to produce more EBs, IM burst to release EBs
- Lacks many genes for metabolic enzymes, making Ct dependent on the host for essential nutrients
- Reroutes Golgi-derived exocytic vesicles as a source of lipids as well as other methods to bolster IM integrity

LDs and *C. trachomatis* infection:
- Host LDs localize to IM as early as 18 hpi
- Located near the cytoplasmic side of the IM and near Ct outer membranes (Kumar et al., 2006)



LDs accumulate at the periphery of the *C. trachomatis* inclusion (Cocchiaro, et al., 2008)

Putative chlamydial proteins that target host LDs:
- Lda1, Lda3- translocate to host cytoplasm then localize to IM cytoplasmic side Lda3 aids in LD-IM fusion by stripping LD of the coat protein ADRP
- IncA marks segments of IM-LD association (Cocchiaro, et al., 2008)
  - Conflict-Saka et al., 2015- no evidence of IncA on LDs in Ct-infected HeLa cells
  - Lda3 and Lda1 localized to LDs only when ectopically expressed
- CT775- membrane-bound chlamydial protein is a putative LPCAT that associates with host LDs
- Human acyl-CoA carrier (hACBD6) moves from nucleus to associate with LDs following Ct infection and is translocated into the IM (Soupene et al., 2014)
  - hABCD6 proposed to modulate the enzymatic activity of CT775- acting together for LD-IM translocation



A model for LD interaction with the *Chlamydia* inclusion. (Cocchiaro, et al., 2008)

Importance of LDs for Ct survival and infectivity:
- Yes- Ct growth is dependent on the presence of LDs, using LD inhibitor triacsin C (Kumar et al., 2006) (Cocchairo et al., 2008)
- For *optimal* growth- mouse embryonic fibroblast (MEF) cells derived from double knockout DGAT1/DGAT2 mice decreased Ct infective progeny (Saka, 2015)
- For *optimal* growth- LD depleted cells have decreased IM size and infectivity but don't require LDs for survival (Recuero, 2016) (Yao, 2015).
- No- Triacsin C has an off-target effect & OA treatment increased inclusion formation in Triacsin C-treated cells
  - negates Kumar and Cocchairo's findings (Soupene et al., 2014) (Sharma et al., 2018)
- ASCLs are translocated into IM for lipid modification independent of LDs (Soupene et al., 2017)

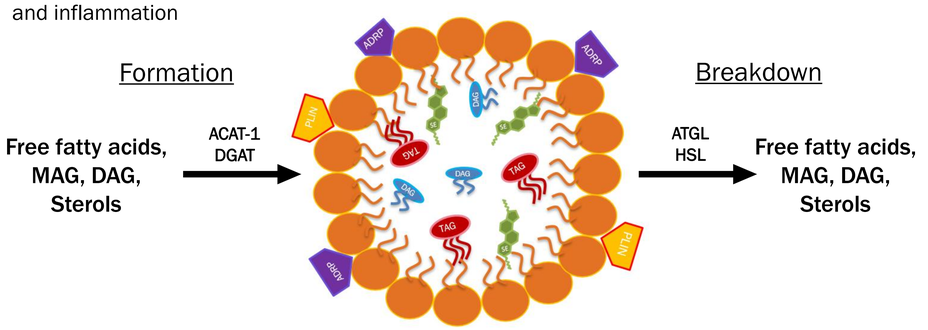
Latest Findings:
- IM size & EB production peaked in WT cells at 24 hpi while LD devoid cells peaked in size & EB production at 48 hpi
- Significant decrease in infectivity of LD depleted cell lines at 24 hpi with much higher metabolic activity
- Infectious progeny in LD depleted cells peaks at 48 hpi
- Fatty acid availability is a better determinate of Ct growth and development (Sharma et al., 2018)

Goal

Summarize the published literature describing the pathways obligate intracellular bacterial pathogens employ to manipulate lipid droplets and identify the contribution of lipid droplets to bacterial intracellular survival and infectivity

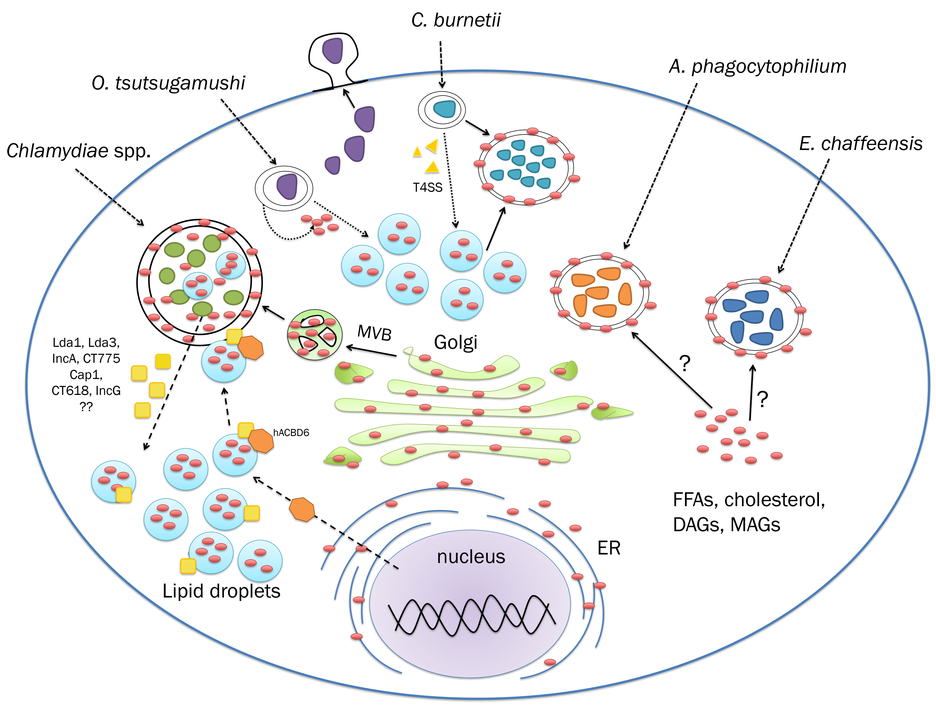
What are Lipid Droplets?

- Cytoplasmic lipid storage organelles surrounded by a phospholipid monolayer
- Store excess cellular free fatty acids and cholesterol as triacylglycerol (TAG) and cholesterol ester (CE) respectively
- Functions include lipid metabolism, energy homeostasis, membrane trafficking, cell signaling, and inflammation



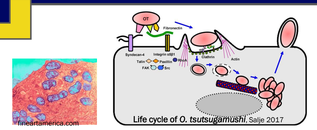
LD= Lipid droplet SE= sterol ester PLIN= Perilipin, LD- bound protein ADRP= Adipocyte Differentiation Related Protein, LD- bound protein
TAG= Triacylglycerol DAG= Diacylglycerol MAG, DAG, TAG — Mono-, Di-, and Triacylglycerol DGAT — Diacylglycerol acyl transferase
ACAT-1 — Acyl coenzyme A acetyl transferase ATGL —Adipose triglyceride lipase HSL — Hormone-sensitive lipase

Conclusion



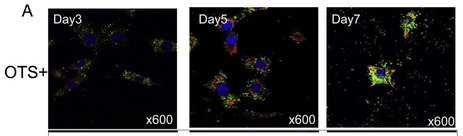
Orientia tsutsugamushi

Causative agent of scrub typhus



Pathogenesis
- Obligate intracellular pathogen
- Infects endothelial cells, macrophages, cardiac myocytes
- Enters host by attaching to integrin α1-β1 and syndecan 4 receptors via surface proteins such as fibronectin and surface cell antigen 7 autotransporter, then internalize through a clathrin-dependent pathway
- Escapes endosomal vacuole and grows in the cytosol

Lipid droplets and *O. tsutsugamushi*
- During multiplication in the cytosol, the bacterium promotes cellular fatty acid esterification, induces an accumulation of triglycerides, and increases LD formation independently from external fatty acids.



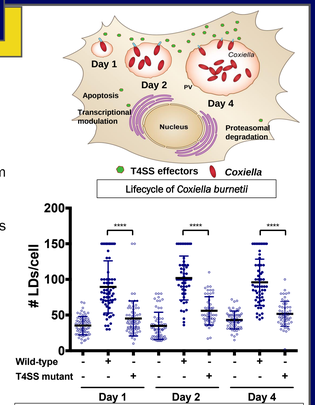
Formation of lipid droplets in mice L-929 fibroblasts infected with *Orientia tsutsugamushi*. (*Ogawa et. al. 2014*).

Coxiella burnetii

Causative agent of human Q fever & endocarditis

Pathogenesis
- Gram-negative, obligate intracellular coccobacillus
- Infects alveolar macrophages
- Grows and replicates inside a parasitophorous vacuole (PV)
- Releases bacterial effector proteins via Type 4 Secretion System (T4SS) to manipulate host cell functions
- T4SS effectors help biogenesis and maintenance of the PV
- Higher cholesterol concentrations on the PV membrane increases acidity within the PV resulitng in bacterial death

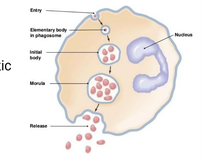
Lipid droplets and *C. burnetii*
- *C. burnetii* induces LD accumulation in alveolar macrophages using the T4SS
- Manipulation of host LD homeostasis alters *C. burnetii* intracellular growth
  - Blocking LD formation increases bacterial growth
  - Blocking LD breakdown inhibiteds bacterial growth
- Suggests that LD lipolysis is vital for *C. burnetii* survival and proliferation in alveolar macrophages



Wild-type and T4SS mutant *C. burnetii*-infected cells were stained for Plin2 and the bacteria. Number of LDs were counted by fluorescence microscopy. Graph represents number of LDs/cell in uninfected and infected cells. (n=3)

Anaplasma phagocytophilium & *Ehrlichia chaffeensis*

Causative agent of human granulocytic anaplasmosis and human monocytic ehrlichiosis, respectively.

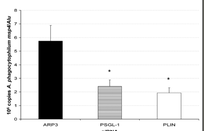


Lifecycle of *Anaplasma* and *Ehrlichia*

Pathogenesis
- Obligate intracellular tick-borne pathogen
- Infects monocytes-macrophages and neutrophils
- Enters cell through caveolae-mediated endocytosis
- Both pathogens exploit caveolae within phagocytes

Virulence factors
- T4SS present may be responsible for the delay in apoptosis of infected host cells

Lipid droplets and *A. phagocytophilium* and *E. chaffeensis*
- Both require maintenance of cholesterol levels for structural integrity, survival, and virulence
- Obtain exogenous cholesterol or its derivatives from the host (Linn, et. al).
- Human promyelocytic HL-60 cells infected with *A. phagocytophilium* revealed that as it multiplies, perilipin mRNA levels are increased and localized in the cytoplasm and periphery, specifically targeting LDs.
- Perilipin knockdown significantly reduces infection (De la Fuente, et. al).



Effect of PLIN knockdown on *A. phagocytophilium* infection of human HL-60 cells. (De la Fuente, et. al, 2008)

Acknowledgements

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